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Letter to the Editor

Low Incidence of Myotonic Dystrophy in Chinese Hans Is Associated With a Lower Number of CTG Trinucleotide Repeats

To the Editor:

Myotonic dystrophy (DM) is an autosomal dominant neuromuscular disease characterized by myotonia, progressive weakness, and wasting of skeletal muscles, as well as a wide range of associated symptoms including cataracts, frontal balding, and intellectual impairment [Harper, 1989]. Although the biochemical mechanism underlying the disorder is still unknown, the genetic defect is believed to be the abnormal expansion of the CTG repeats in the 3' untranslated region of the myotonin protein kinase (DMPK) gene located on chromosome 19q13.3 [Buxton et al., 1992; Harley et al., 1991, 1992]. In normal individuals, the range of the triplet repeat number falls between 5 and 35 and is variable among different populations. In patients, the repeats are usually greater than 50 and may be as high as 2,000 in severely affected cases. The number of the repeats correlates well with the age of onset and severity of the disease within families [Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992].

Estimates of the frequency of DM in global populations vary widely, from 1 in 475 in certain regions of Canada [Bouchard et al., 1989] to 1 in 8,000 overall for Western European and North American populations [Harper, 1989]. In Japan, one in 20,000 is estimated to be affected [Osame et al., 1983], while in sub-Saharan Africa only one Nigerian affected family has been ever reported [Dada, 1973]. In China, myotonic dystrophy is scarce. Up to 1988, in total only about 100 cases were reported [Liu and Tang, 1988] and no DM patient was discovered in a general population of 126,876 people during an epidemiological survey of inheritable diseases in Sichuan Province [Zhang et al., 1990]. Also in a neurogenetic-epidemiological survey covering 152,818 people in Guangdong Province, no DM case was found [Liu et al., 1985].

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Our previous study and a study by another group on CTG repeat polymorphism [Pan and Zhang, 1996; Tishkoff et al., 1998] found 14 and 7 alleles in 159 and 42 Chinese, respectively, and most studies on different normal populations revealed an overall trimodal distribution of the CTG repeat alleles with modes corresponding to 5, 11–17, and 19–31 CTG repeats. At the same time evidence has been accumulated that the CTG allelic distribution may explain, at least in part, the variation in frequencies of DM in different ethnic populations [Goldman et al., 1994; Krahe et al., 1995; Zerynick et al., 1995]. To prove this, we expanded our previous study on the CTG repeat polymorphism in normal Hans who constitute 95% of the Chinese population and compared the allele distribution with the data from Caucasian, Japanese, and Southern Africa Negroid populations in connection with their incidence of myotonic dystrophy.

Three hundred unrelated Chinese Hans aged from 20 to 45 years from Chengdu, the capital city of Sichuan Province, were studied. Genomic DNA was prepared from peripheral blood cells by using a phenol-chloroform extraction procedure. PCR analysis of CTG repeat size (Fig. 1) was carried out with primers described by Guida et al. [1995]. Then the PCR products were separated on 6% non-denaturing polyacrylamide gel by using DNA samples of known CTG repeat number estimated by sequencing analysis and commercial DNA markers as standards of the allele size. Statistical significance was determined by using the χ^2 test with the Yate's correction when necessary.

The variation in CTG repeats of 600 normal chromosomes is shown in Figure 2 and Table I. A total of 16 alleles ranging in size from 5 to 29 repeats are found. The most common allele has 5 repeats (35.67% of chromosomes) and the majority of alleles are in the range of 11 to 13 repeats (51.50%) with 12 repeats being more frequent (18.67%). Only two chromosomes are observed with 6 and 8 repeats, respectively, and very few alleles are found with more than 14 repeats (5.0%). It is significant that only 6 alleles (1.0%) are found with 19 repeats or more.

Comparisons of allele frequencies from present study with those in European, Japanese [Davies et al., 1992] and Southern African Negroid [Goldman et al., 1994] populations has shown (Fig. 2, Table I), beside varia-

426 Zhang et al.

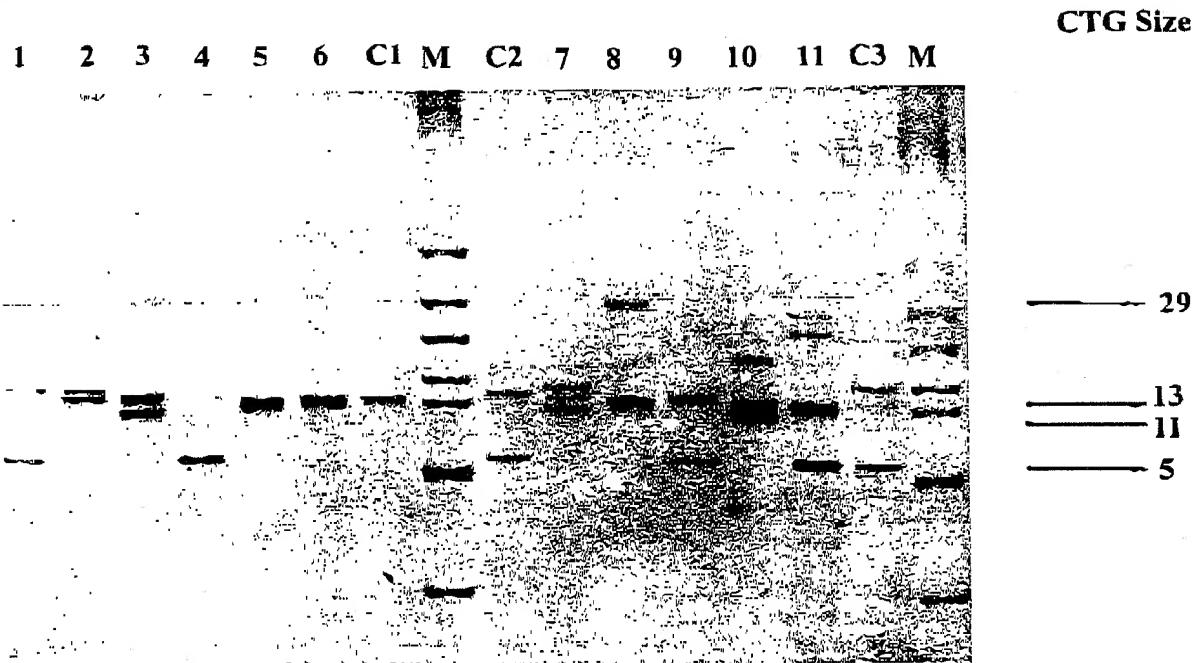


Fig. 1. DMPK CTG-repeat size in Chinese Han population. PCR amplification was carried out as described and products were resolved on a 6% polyacrylamide gel. Alleles were sized by capillary electrophoresis of known size PCR products and pBR322/MuclII marker. Numbers indicate individual Han samples. M, DNA marker; Cs, known size PCR products.

tions in frequencies of some alleles among populations, the different frequencies of alleles with larger number CTG repeats. When using 19 repeats as the cut off point, the frequencies of alleles with $(CTG)_{\leq 19}$ are 10%,

8.5%, 0.7%, and 1.0 % in Caucasian, Japanese, Southern African Negroid, and Chinese Han, respectively. The difference is highly significant between Chinese and Caucasian, Chinese and Japanese ($\chi^2=21.7$, P

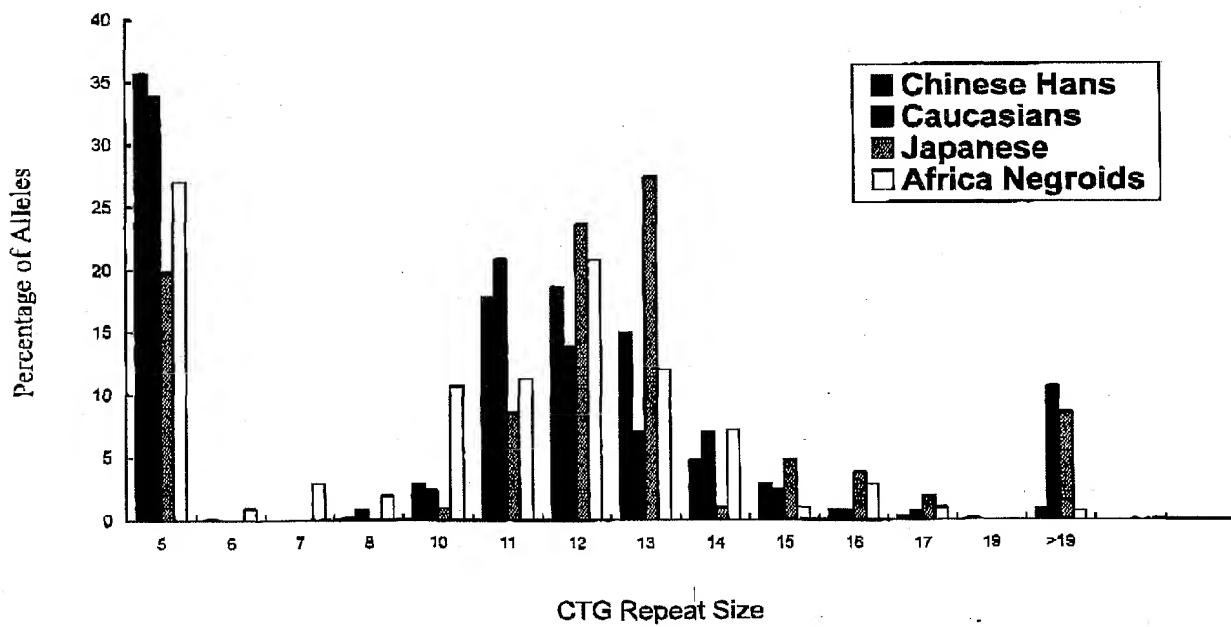


Fig. 2. Distribution of DMPK CTG repeat alleles in Chinese Han samples (black bars), Caucasians (lattice bars), Japanese (slashed bars), and Southern African Negroid (blank bars) populations.

Letter to the Editor

427

TABLE I. Distribution of CTG Repeat Alleles in Chinese Han (C), European (E), Japanese (J), and Southern African Negroid (N) Populations With χ^2 Test and Yates' Correction When Necessary

Number of CTG repeats	Number of chromosomes			P value			
	European ^a	Japanese ^a	Negroid ^b	Chinese	CvE	CvJ	CvN
5	44	21	112	214	0.77	0.02	0.003
10	3	1	45	17	0.78	0.33	0.00001
11	27	9	47	107	0.45	0.02	0.004
12	18	25	87	112	0.21	0.28	0.42
13	9	29	50	90	0.01	0.002	0.17
14	9	1	33	28	0.87	0.11	0.0047
15	4	5	4	17	0.89	0.47	0.063
16	1	4	12	5	0.64	0.043	0.025
17	1	2	3	2	0.96	0.21	0.69
≥ 19	13	9	3	6	0.000	0.000	0.888
Total	180	106	420	600			

^aDavies et al. [1992].^bGoldman et al. [1994].

<10⁻⁴ and $\chi^2 = 14.7$, $P < 10^{-3}$, respectively). Thus, the Chinese Han is significantly different in this respect from the allele distribution of Caucasian and Japanese populations. Meanwhile it is very similar to that of Southern African Negroid where a paucity of larger repeats is also observed. No allele longer than 35 CTG repeats has been found in any of the four groups.

The present study strongly suggests that the larger CTG number, although still within the normal range, may have a greater chance of progressing into the pre-mutation and mutation range of repeats and hence to cause DM in the next generation. The findings of Imbert et al. [1993] also suggest that the CTG repeat is the main driving force for DM gene mutation and alleles with greater than 19 CTG repeats are more unstable and thus may be the predisposing factor for DM mutations. Other population studies also provide evidence for the association between disease prevalence and CTG copy number [Tishkoff et al., 1998]. In European populations, where the frequency of alleles with $(CTG)_{\geq 19}$ is 10 %, the frequency of DM is 1/8,000, while in Southern African Negroid, for the frequency of alleles with $(CTG)_{\geq 19}$ is 0.7%, only one family of DM has been so far reported.

In China, myotonic dystrophy is a very rare disease and, from present studies, the alleles with CTG repeats greater than 19 are only 1%. This may explain, at least partially, the low incidence of DM in Chinese Hans and provides further evidence for the association of disease incidence with the frequency of alleles with higher (CTG) repeats in populations.

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428 Zhang et al.

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